

# UNCLASSIFIED

<b>AD NUMBER</b>
AD903316
<b>NEW LIMITATION CHANGE</b>
<b>TO</b> Approved for public release, distribution unlimited
<b>FROM</b> Distribution limited to U.S. Gov't. agencies only; Proprietary Info.; Jun 72. Other requests for this document must be referred to Commanding General, Army Medical Research and Development Command, Attn: SGRD-IDS. Washington, D. C. 20314.
<b>AUTHORITY</b>
US Army Med Research and Mat Cmd, MCMR-RMI-S, memo dtd 6 Jun 2000, Dep Ch Staff Info Mgt, Ft Detrick, MD. 22060-6218.

THIS PAGE IS UNCLASSIFIED

AD903316

AD

SUSTAINED PROTECTION AGAINST SUPERFICIAL BACTERIAL AND  
FUNGAL INFECTIONS BY TOPICAL TREATMENT (U)

ANNUAL PROGRESS REPORT

by

Albert M. Elizman, M.D.

and

Richard R. Murphy, B.M.

August, 1972

(For the period 1 September 1971 to 31 May 1972)

Supported by

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND  
Office of the Surgeon General, Washington, D.C. 20314  
in cooperation with the Commission on Cutaneous Diseases  
of the Armed Forces Epidemiological Board

Contract No. DADA-57-11-0-1000

University of Pennsylvania  
Philadelphia, Pennsylvania 19104

DISC DISTRIBUTION STATEMENT

Distribution limited to U.S. Government agencies only.

~~For release by the Department of Defense, June 1972. Other requests for~~

~~this document will be referred to the Commanding General,~~

~~Fort Detrick, Research and Development Command, Fort Detrick,~~

~~Research Triangle Park, North Carolina 27709.~~

~~For release by the Department of Defense, June 1972. Other requests for~~

~~this document will be referred to the Commanding General,~~

~~Fort Detrick, Research and Development Command, Fort Detrick,~~

~~Research Triangle Park, North Carolina 27709.~~

~~For release by the Department of Defense, June 1972. Other requests for~~

~~this document will be referred to the Commanding General,~~

~~Fort Detrick, Research and Development Command, Fort Detrick,~~

~~Research Triangle Park, North Carolina 27709.~~

~~For release by the Department of Defense, June 1972. Other requests for~~

~~this document will be referred to the Commanding General,~~

~~Fort Detrick, Research and Development Command, Fort Detrick,~~

~~Research Triangle Park, North Carolina 27709.~~

~~For release by the Department of Defense, June 1972. Other requests for~~

~~this document will be referred to the Commanding General,~~

~~Fort Detrick, Research and Development Command, Fort Detrick,~~

~~Research Triangle Park, North Carolina 27709.~~

AD \_\_\_\_\_

**SUSTAINED PROTECTION AGAINST SUPERFICIAL BACTERIAL AND  
FUNGAL INFECTIONS BY TOPICAL TREATMENT (U)**

**ANNUAL PROGRESS REPORT**

by

**Albert M. Kligman, M. D.**

and

**Richard R. Marples, B. M.**

**August, 1972**

**(For the period 1 September 1971 to 31 May 1972)**

**Supported by**

**U. S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND  
Office of the Surgeon General, Washington, D. C. 20314  
in cooperation with the Commission on Cutaneous Diseases  
of the Armed Forces Epidemiological Board**

**Contract No. DADA-17-71-C-1009  
University of Pennsylvania  
Philadelphia, Pennsylvania 19104**

**DDC DISTRIBUTION STATEMENT**

**Distribution limited to U. S. Government agencies only,  
Proprietary Information. June 1972. Other requests for  
this document must be referred to the Commanding General,  
U. S. Army Medical Research and Development Command**

**The findings in this report are not to be construed as  
an official Department of the Army position unless so  
designated by other authorized documents.**

### ABSTRACT

The feasibility of providing sustained protection of the skin against infection by common micro-organisms has been shown in previous reports. Some anti-microbial agents remain active and persistent on human skin. Neomycin reduces growth of the flora under occlusion at a dose of 0.4 micrograms per sq. cm. Neomycin, chloramphenicol and some other drugs will reduce the high numbers of organisms created by preocclusion.

Experimental infections with Staphylococcus aureus on mildly traumatized skin have been extensively investigated. By delaying inoculation of the pathogen until 24 hours after injury a safe yet realistic infection can be produced. The requirements for a valid test of antibacterial action are still in doubt but the ability of the test to detect effective antibacterial action is not doubted. Similarly basic studies of the Candida albicans infection model have indicated the safety repeatability and practical value of this test. Infections with Pseudomonas can now be created but repeatability remains a problem.

## Contents and Illustrations

Introduction .....	p. 4
Prevention of overgrowth of normal flora by neomycin .....	p. 5
Figure 1	
Pre-occlusion .....	p. 6
Table 1	
Persistent anti-bacterial action of chloramphenicol .....	p. 7
Table 2	
Experimental infections with Staph. aureus on stripped skin .....	p. 8
Bacteriological and Clinical Effects in Stripped Sites .....	p. 9
Experiment 1 .....	p. 10
Experiment 2 .....	p. 11
Figure 2	
Experiment 3 .....	p. 12
Table 3	
Studies on experimental candidiasis .....	p. 13
Severity of reaction .....	p. 14
Figure 3	
Dynamics of the population in the first 24 hours .....	p. 15
Figure 4	
Interdigital infection .....	p. 16
Figure 5	
Figure 6	p. 17
Pseudomonas infections .....	p. 18
Table 4 .....	

## INTRODUCTION

Microbial infections of the skin, both primary and secondary, are a major cause of military non-effectiveness. The purpose of this project is to develop a formulation for topical application which could prevent bacterial and fungus infections for a period of days after application.

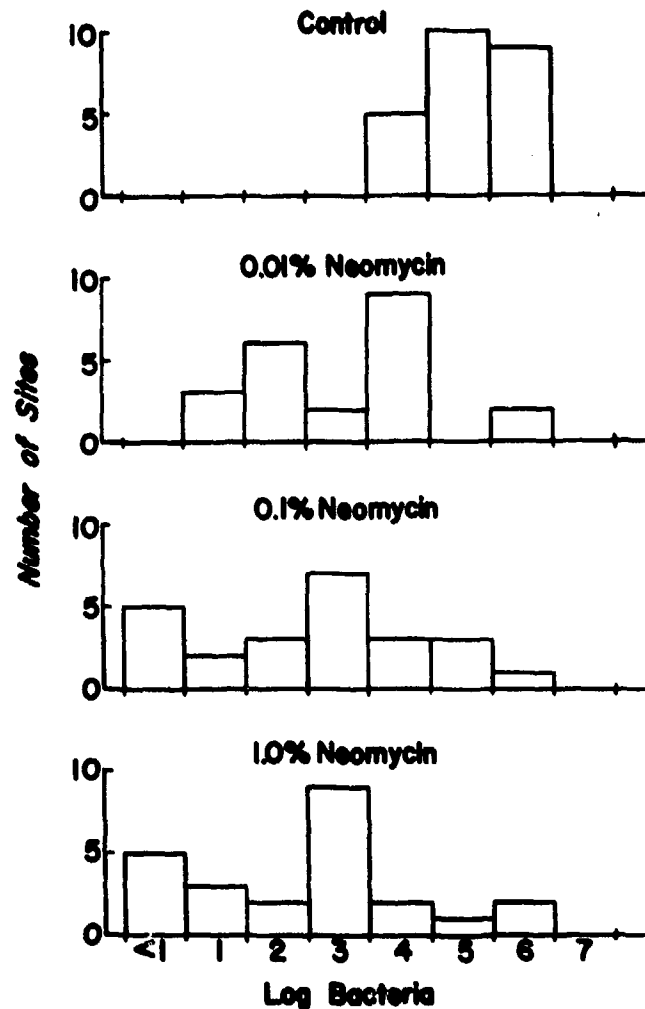
Previous reports have indicated that several antibiotics and antibacterial agents possess the attributes of effectiveness on the skin and persistence of the effects for several days. The list includes:

Bexide  
Bronopol  
Chloramphenicol  
Demeclocycline  
Erythromycin  
Hydroxyquinolinol  
Irgasan CH3565  
Neomycin  
Penicillin G  
Pyridine Thiones

These and other reference antimicrobials form the basis of the study.

In this report we present some comparative results of efficacy and dosage for these agents and methods for testing against experimental infections with Staphylococcus aureus, Candida albicans and Pseudomonas.

### Effect of Neomycin on Bacterial Density in the Occlusion Test



#### Prevention of overgrowth of normal flora by neomycin

In 24 volunteers four 5 cm squares were marked out on the forearm. Three received 0.1 ml of 1% , 0.1% or 0.01% aqueous solution of neomycin sulphate. A no-treatment control was included. Each site was covered with a 5 cm square of polyethylene film for 24 hours and then sampled quantitatively with the detergent scrub technique.

**Results:** Figure 1 shows the density of organisms for each dilution. In most instances all 3 concentrations were highly effective in preventing overgrowth. However, 0.01% was somewhat less effective than 0.1%. The amount of drug actually present at the lowest concentration is 0.4 ug/cm<sup>2</sup>. These results make it clear that neomycin has potent anti-bacterial activity on human skin.

### Pre-occlusion

The amount of drug required to prevent the overgrowth of normal skin organisms can be determined and permits comparisons to be drawn between different antimicrobial agents. However, in the persistence tests previously reported, the drug was merely required to prevent the resident flora from increasing in numbers. In the new procedure, a huge population is induced by occlusion and the capacity of the agent to eradicate this mixed flora is assessed.

**Methods:** Both forearms of volunteers were wrapped in polyethylene film for 48 hours. This resulted in a large number of bacteria, of the order of  $10^6$  per sq. cm. As the dressings were removed four 5 cm. squares were treated with 0.1 ml of 1%, 0.1% or 0.01% of the agent in water or ethanol containing 10% isopropyl myristate or the vehicle alone. Each site was covered with a polyethylene film square held in place with adhesive tape for 24 hours. The sites were then sampled with RODAC contact plates containing trypticase soy agar with lecithin and polysorbate 80 as neutralizers. Density was recorded on the following scale:

0 less than 10 colonies  
+ 10-30 colonies  
++ 30-300 colonies  
+++ some colonies touching  
++++ most colonies touching  
+++++ confluent growth

**Results:** Eleven different compounds were tested in groups of 5 volunteers. The average score is tabulated in table 1.

Table I  
Average score on contact plates when antimicrobial agents are applied to a dense flora

	1%	0.1%	0.01%	Control
Bexide	2.8	4.3	2.5	3.3
Bronopol	2.0	4.0	4.8	4.5
Chloramphenicol	1.5	0.8	2.8	5.0
Erythromycin	2.6	4.2	4.2	5.0
Hexachlorophene	4.6	4.6	5.0	4.8
Neomycin	1.3	1.0	3.5	4.3
Penicillin G	5.0	5.0	5.0	5.0
Pyridinethione sodium	3.8	4.4	5.0	5.0
Pyridinethione zinc	2.4	3.6	4.4	4.8
Tetracycline base	3.0	3.3	5.0	5.0
Tetracycline HCl	3.0	4.0	3.8	5.0



It can be seen that even at 0.01% chloramphenicol, neomycin and tetracycline have activity while Bexide is doubtful. Even at 1% hexachlorophene and penicillin G appear essentially inactive. Overgrowth of gram negatives is responsible for this apparent paradoxical finding in the case of hexachlorophene.

Comments: This method of assaying the activity of an antimicrobial is not sufficiently quantitative since a relatively few resistant organisms will overgrow contact plates. This was quite evident with erythromycin at 0.1% and 0.01% which lacked effectiveness because of overgrowth of Proteus. Dilution techniques and colony counting should overcome these limitations.

#### Persistent anti-bacterial action of chloramphenicol

Chloramphenicol was dissolved in a concentration of 5% in a solution of 70% ethanol, 10% propylene glycol, and 20% water.

The persistence of an antimicrobial effect after a single application was determined on successive days by occlusion for 24 hours and quantitative sampling.

Table 2  
Persistence of Anti-bacterial activity after a single application of chloramphenicol

Bacterial Counts per sample				
Immediate	1 day	2 days	3 days	
40	0	3 200	800	
10	3 600	80	1 120	
10	40	160	32 000	
56 000	40	920	320 000	
10	40	800	28 000	
270	230	0	56 000	

Results: For at least 48 hours chloramphenicol exerted a more or less complete suppressive effect. Very few organisms could be recovered. Suppression had begun to wane by 72 hours but there was still considerable anti-bacterial activity. Other studies in progress indicate that curtailment of bacterial growth may last for 5 days following a single application of chloramphenicol. These and other data make chloramphenicol an exceptional drug. We plan more intensive studies.

### Experimental infections with Staph. aureus on stripped skin:

Our objective was to develop a realistic human model for studying infections of superficially wounded skin. We elected to use the technique of stripping away the horny layer barrier with cellophane tape - a simple procedure which has been of inestimable value in dermatologic investigations of many kinds. It is important to realize that stripping does more than remove the stratum corneum; it damages the viable epidermis much more than originally believed. A burst of mitotic activity occurs which reaches a peak at about 48 hours. Diffusional water loss is very high but the formation of a parakeratotic layer provides a temporary barrier which even 24 hours later is much less permeable. The anatomic regeneration of a new horny layer takes about 10 days.

### Methods

Stripping: Up to four sites were marked out on the forearm and the stratum corneum removed with cellophane tape until the glistening layer was reached. Two assistants alternately applied 1 inch wide strips of cellophane tape at right angles preparing thereby a square area with a moist surface. The strips were firmly applied and briskly ripped off since we wished to promote transudation of serum. A period of twenty-four hours was usually permitted to elapse before inoculation.

Inoculation: A non-typable skin strain of S. aureus susceptible to all common antibiotics was used throughout. Cells from an overnight surface growth on Trypticase Soy Agar were suspended in saline to form a turbid suspension. The number of viable cells per ml. was established by counting in culture.

A volume of 0.01 ml. was applied by micropipette to the stripped skin which was then covered with a 2 cm. square of polyethylene film sealed to the skin under adhesive tape.

Cytological Study: After 24 hours of occlusion clean slides were applied to the stripped surface or to the contact side of the polyethylene film. The limits of the area under plastic film were marked on the back of each slide which was stained with May-Grunwald-Giemsa for study of leukocytes and bacteria.

Clinical Evaluation: The sites were scored on a 5-point scale based upon the intensity of erythema and suppuration twenty-four hours after inoculation. The estimates were in relation to the appearance of uninoculated stripped sites which were somewhat reddened and moist but usually not suppurative. All but the mildest infection evoked a thin serosanguineous exudate over a strongly erythematous base. Grade 5 infections showed ulceration and extension beyond the inoculation site.

## Results

### Bacteriological and Clinical Effects in Stripped Sites

Uninoculated Sites: When left unoccluded resident organisms, chiefly coagulase negative staphylococci, were present on the stripped site but in lower numbers than the surrounding normal skin only in the first 24 hours. Microbial numbers then rose above control levels by the second or third day and thereafter slowly declined. Rarely, perhaps once in fifty subjects a spontaneous S. aureus infection occurred. In the absence of infection the sites rapidly lost redness, developed a brownish leathery crust and healed without scarring.

When a site stripped one day earlier was occluded without inoculation its appearance 24 hours later showed a mild reddish-brown color and a moist shiny surface. Again, and somewhat more frequently, spontaneous infection could occur during this 24 hour period. S. aureus was the usual invader but various enterobacteria sometimes became established. Spontaneous infections were much more frequent when uninoculated stripped sites were occluded immediately after stripping. Twenty-six sites were studied in this way. S. aureus became dominant in 5 and was recovered in moderate numbers in another five sites. Enterobacteria dominated the flora in one site and were present in 4 others. Pseudomonas was recovered in moderate numbers from one site. Coagulase negative staphylococci were the dominant organism in 20 cases. In this series clinically apparent infection (grade +++) occurred but once in a site which yielded  $8 \times 10^6$  S. aureus per sq. cm. and no other organisms.

Occlusion immediately after stripping thus sometimes led to abnormal bacterial populations and to actual clinical infections. By delaying the occlusion for one day, there was uneventful recolonization on the site by resident skin organisms. As a consequence, uninoculated stripped sites occluded 24 hours later could serve as negative controls.

Inoculated Sites: S. aureus produced characteristic clinical changes within 24 hours when sites were inoculated and occluded one day after stripping.

Most sites were distinctly moist. A visible exudate, usually serosanguineous, but occasionally purulent was also present. In mild infections, touch smears were more informative of the presence of numerous leukocytes than naked eye evaluation. In more severe infections the exudate was often purulent and copious enough to seep out around the occlusive dressing. Edema, was not prominent. It was our practice to treat each site with a neomycin cream (Neosporin<sup>®</sup>) twice daily for 2 days.

When occlusion was maintained for 2 days instead of one the lesion usually did not become much more inflamed. Nonetheless, severe ulcerative infections occurred in 2 subjects requiring oral antibiotics.

Bacteriology of Inoculated Sites: After 24 hours of occlusion, the density of *S. aureus* was quite high, more than  $10^6$  per sq. cm. Coagulase negative cocci and enterobacteria were occasionally present.

Cytological findings: Three patterns could be recognized in touch slides:

1. The usual picture was a microscopic exudate of polymorphonuclear leukocytes and many cocci whether or not frank pus was evident clinically. Phagocytosis was very evident and the leukocytes were rather well preserved. Some proteinaceous amorphous material was present. This picture was interpreted as a balance between host and pathogen though one or the other might be dominant in some instances.
2. Less commonly, the smear showed innumerable bacteria with a few degenerated granulocytes and considerable amorphous material. An occasional macrophage could be recognized. This was the typical picture with a 48-hour delay before inoculation. It may be interpreted as an overgrowth of bacteria in a privileged situation more or less inaccessible to phagocytes. The host remains indifferent.
3. In the sites showing the greatest signs of inflammation with a copious exudate, the cytologic sence consisted of many leukocytes and few organisms. The leukocytes were excellently preserved and a few red cells could sometimes be identified. This is the picture of a successful response on the part of the host which has vanquished the bacterial invader.

#### Experiments

Experiment 1-Inoculation Immediately after Stripping. In 5 subjects  $10^5$  cells were applied under occlusion to freshly stripped sites. Within 6 hours two of these subjects complained of pain and swelling associated with slight fever. Palpable nodes were present in the axilla. The skin around the inoculum site was hot, reddened and sore. Clinical signs of a spreading cellulitis were apparent in all five. Systemic penicillin was immediately administered and the occlusive dressings removed. This brought about immediate regression of the signs and symptoms.

On a later occasion, a similar situation arose by accident. Again all five subjects showed clinical signs of spreading infection within hours, requiring intercession as above.

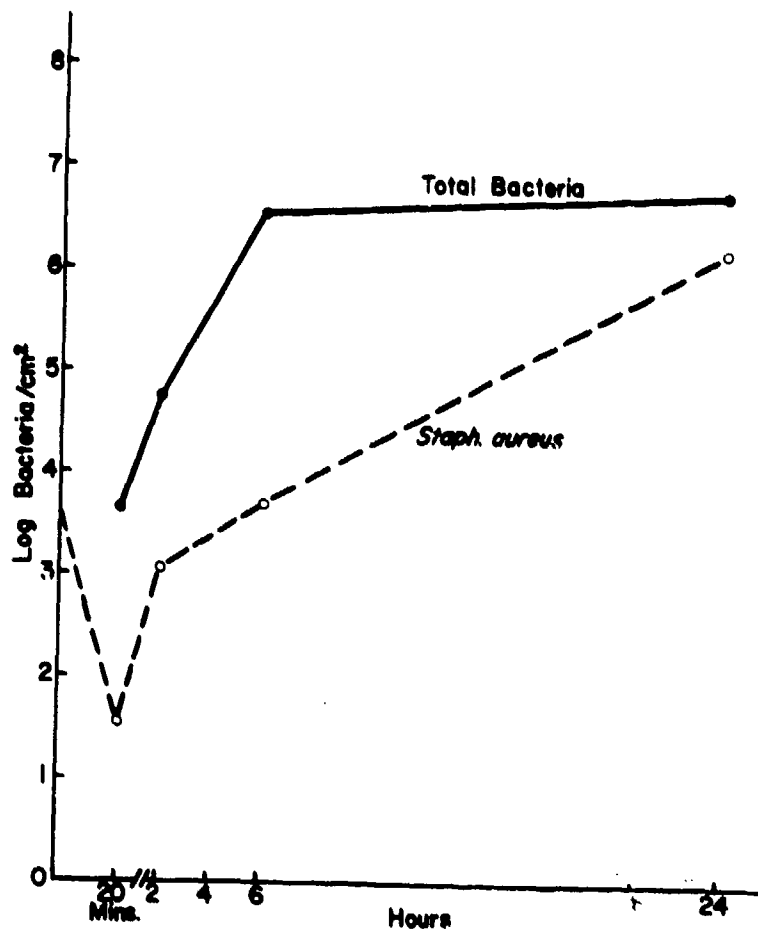


Fig. 2.

Growth of *S. aureus* and resident organisms on occluded stripped sites. Note early decrease followed by steady increase.

Experiment 2-Dynamics of Early Colonization. In 5 subjects 4 stripped sites were prepared and inoculated with 4,000 *S. aureus* cells per sq. cm. after a 24-hour delay. One site each was sampled at 20 minutes and at 2, 6 and 24 hours. The density of all organisms, *S. aureus* and residents, at these various times is shown in Figures 13-5. After 20 minutes, only low numbers were recovered. The total was 4700/cm.<sup>2</sup> of which 360/cm.<sup>2</sup> were *S. aureus*. By 2 hours, the total population was 57,300 and *S. aureus* had reached 1150 organisms per sq. cm. At 6 hours, the density reached 10<sup>8</sup>/cm.<sup>2</sup> in 3 subjects, 2 of whom carried 10<sup>7</sup> *S. aureus*. The geometric mean, however, was 3.53x10<sup>6</sup> organisms of which *S. aureus* made up only 5700. At 24 hours, all 5 sites yielded more than 10<sup>6</sup> cells with a geometric mean density of 5.84x10<sup>6</sup>. More than 10<sup>6</sup> *S. aureus* were present in 4 of the 5 sites with a mean density of 1.64x10<sup>6</sup> cells per sq. cm. The exudate corresponded closely to the microbial density. Foci of polymorphonuclear leukocytes were detected microscopically in 3 subjects - in one of whom pus was present clinically at 6 hours and in 3 to 24. Two of these bore more than 10<sup>7</sup> *S. aureus*. In one subject a copious exudate was associated with only 10<sup>5</sup> *S. aureus*.

Table 3  
Prophylaxis of *S. aureus* infections by Antibiotic Cream

	Neomycin-gramicidin	Cream Base	Untreated
Number of sites	13	13	13
<i>S. aureus</i> positive	5	13	13
Mean density (positive sites)	$1.09 \times 10^4$	$7.41 \times 10^6$	$4.36 \times 10^6$
Cytological exudate	1	8	10
Clinical severity	1.2	2.3	3.0

Experiment 3-Prophylaxis by Topical Antibiotic. An antibiotic active in the presence of skin secretions should prevent multiplication of *S. aureus* and forestall infection.

Three 24-hour - old stripped sites on the forearms of 13 volunteers were inoculated as above with  $4 \times 10^5$  *S. aureus* cells. Six hours later one site was treated with a neomycin-gramicidin cream, the second with the cream base and the third was an untreated control. The sites were occluded for a further 18 hours before sampling with the detergent scrub technique.

*S. aureus* was recovered in huge numbers from all 13 sites which were not treated or those which received the cream base. The geometric mean density was of the order of millions per sq. cm. The antibiotic treated site, however, yielded *S. aureus* only five times in quite low numbers. Microscopically a leukocytic exudate was present in 10 of 13 untreated sites, 8 of the cream base sites but in only 1 site treated with the antibiotic.

#### Discussion

The results indicate that infection with *Staphylococcus aureus* can be induced by inoculating tape stripped skin with relatively few organisms.

The following guidelines are suggested for utilizing this model. Strip smartly to the glistening layer, wait 24 hours and apply  $10^3$ - $10^5$  organisms. Occlude for 24 hours and terminate. Simple removal of occlusion will generally suffice. It is the better part of discretion, however, to apply an antibiotic cream twice daily for the next two days. We have routinely used a neomycin cream.

Though safety is the compelling factor in terminating the infection after 24 hours, one usually has all the desired data by that time. Several kinds of appraisal are possible, clinical, bacteriological and cytological. Clinical appearance only may be misleading since true infections may be overlooked if the exudate is not voluminous and all three methods are complementary. Touch plates are not troublesome and rather reliably indicate the amount of bacterial growth particularly when antibacterial agents are being studied.

The immediate uses for this model are to study the pathogenesis of pyogenic infections and to appraise the effectiveness of topical or systemic antibiotics in superficial S. aureus infection. Important measures of host resistance may be determinable with this technique including the effects of underlying disease (diabetes, lymphoma), sex, age, race, etc. on natural resistance.

### Summary

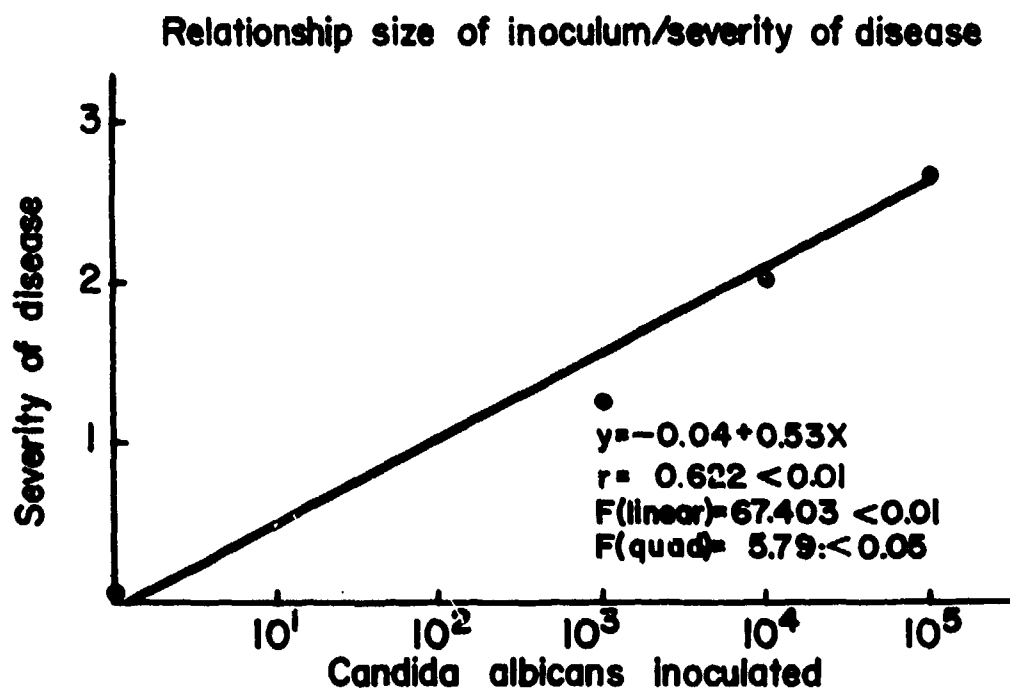
A human model has been developed for reating S. aureus infections after superficial wounding. The technique entails the application of a few thousand organisms to skin stripped of its horny layer twenty-four hours earlier. The site is then covered with an impermeable dressing for 24 hours. This results in a bright red, exudative lesion which generally shows great numbers of leukocytes and bacteria; the density of the latter commonly exceeds  $1 \times 10^6/\text{cm}^2$ .

The high proportion of takes with small numbers of organisms, 100 or less, is attributable to four conditions which stripping peculiarly provides: moisture, serum nutrient, absence of phagocytes during rapid growth and few competing organisms.

### Studies on experimental candidiasis

In previous studies of Candia infections from this laboratory, the inoculum was not precisely known. We have expanded our studies of the pathogenesis of Candida infections using quantitative techniques.

Results: When an inoculum of  $10^5$  cells is occluded for 24 hours there is little or nothing to be seen in 90% of patients at the time of removal of the dressings. Over the next 24 hours a dermatitis limited to the occluded area develops. Initially there is erythema, then pin head vesicles appear which evolve into pustules. In severe cases rupture of the vesicles leads to erosions. This is inevitable if occlusion is prolonged over 24 hours. Patients do vary somewhat in the time of appearance of lesion.



**Fig. 3.**

Relationship of clinical reaction to dose of *C. albicans*. The dosage response is linear.

**Severity of reaction:** The severity of the dermatitis was a linear function of the log of the number of organisms inoculated as was the number of organisms present at the end of the 24 hour occlusion period (Fig. 3 and 4). The equation for the dose response curve was found to be:

$$\text{Severity} = -0.04 + 0.53 (\log \text{Candida inoculated})$$



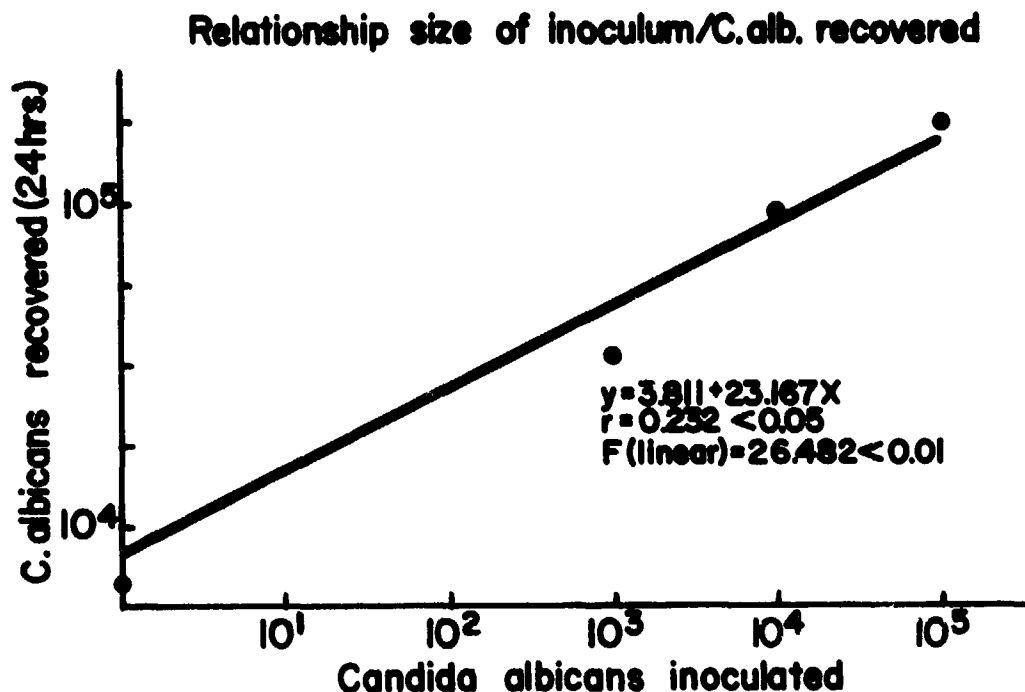


Fig. 4.

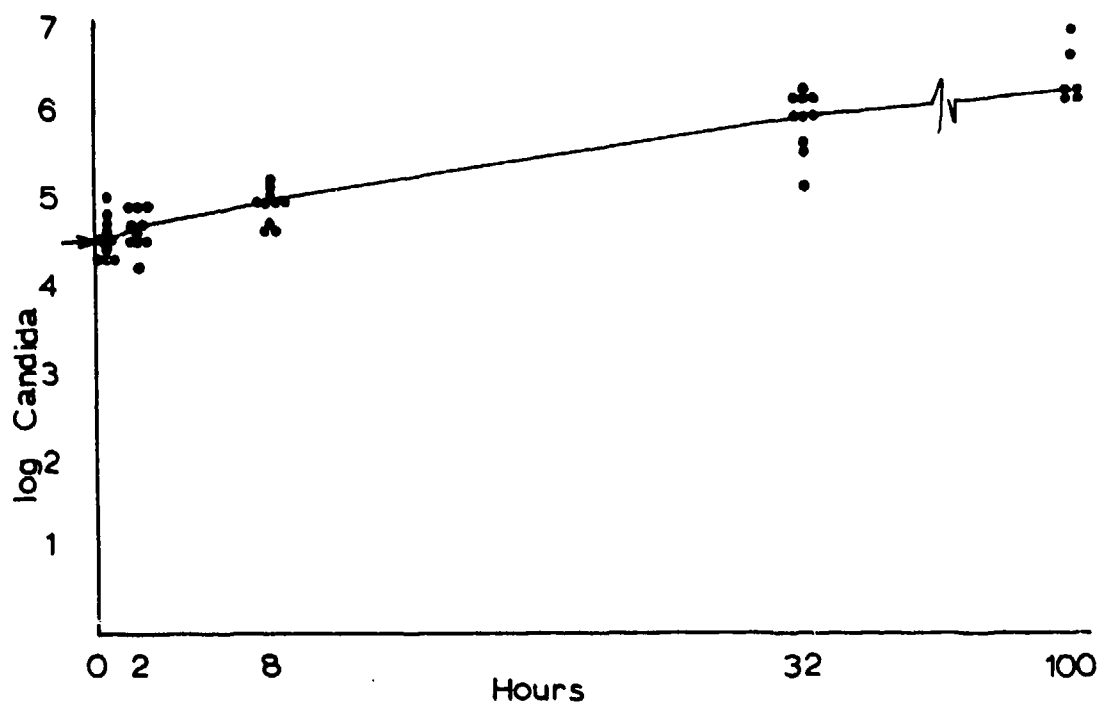
Recovery of C. albicans after various inoculums. The quantity recovered is proportional to dose.

Dynamics of the population in the first 24 hours

In 10 subject 5 sites were inoculated with  $10^5$  cells. One site was sampled after 30 minutes, 2, 8, 32 and 107 hours. The number of mycelial elements was assessed by direct microscopy as well as determining viable cells by culture.

After a lag phase the density of C. albicans increased linearly for 32 hours then tended to level off although still increasing. (Fig. 5.). The number of mycelial elements increased also but the proportion fell 32 hours and then remained stable (Fig. 6.).

These findings do not support the generally accepted view that only the mycelial phase of C. albicans is pathogenic.



**Fig. 5.**

Multiplication of *C. albicans* on occluded normal skin.

**Interdigital infection:**

Merely by taping the knuckles of the middle and ring fingers together with a narrow strip of tape the interspace becomes a suitable site for colonization by *C. albicans*.

By the third day after inoculation of  $10^5$  yeast cells a dermatitis appeared.

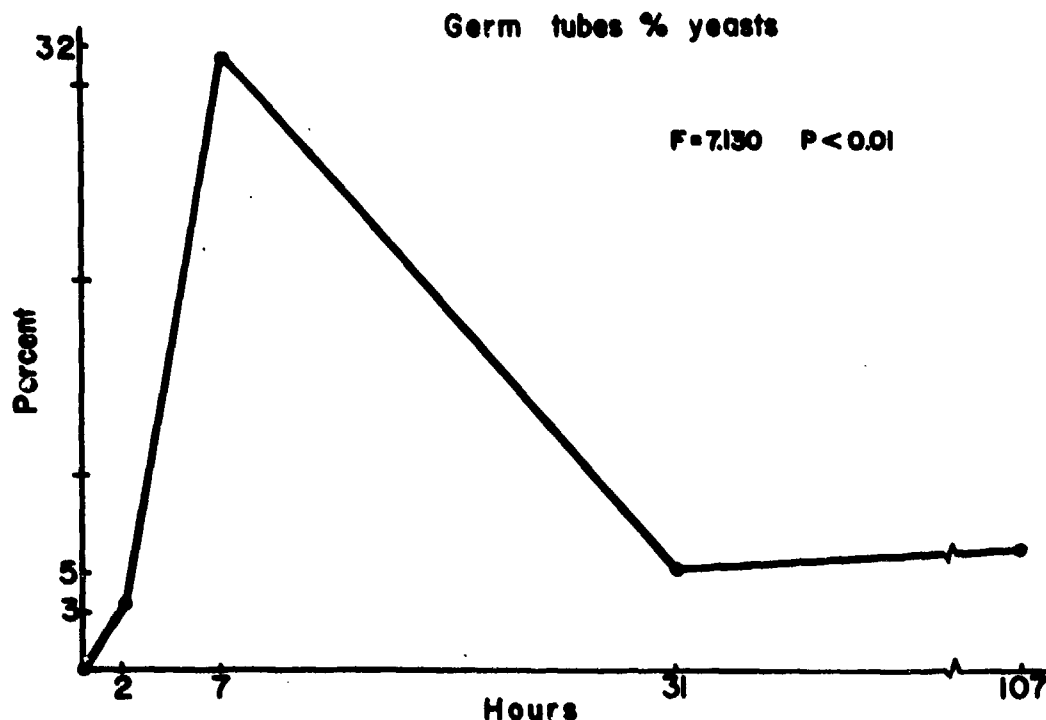


Fig. 6.

Percentage of mycelial elements in developing candida infections.

This was initially erythematous but pustules emerged which left a weeping, red, eroded surface. This was surrounded by satellite lesions. The appearance is identical with the clinical condition *erosio interdigitale blastomycetica*. Non-inoculated fingerwebs showed only the changes of maceration with no sign of erosion or pustulation.

Of particular interest was the finding that enteric bacteria colonize the monilial lesion and, since they may amount to more than 60% of the flora, may make the detection of *C. albicans* more difficult. It is not possible to assert that the final clinical lesion is due only to the yeast. Gram negatives may play some role in the perpetuation of this chronic lesion.

This model will be extremely useful in assessing the value of antimicrobial agents since it is easy to induce yet complex in aetiology. Preliminary studies indicate that agents which are solely anti-fungal are not curative once the lesion is established.

Table 4  
Density of *Pseudomonas*

Subject	Wet Cotton Felt			Polyethylene Film		
	4 days	6 days	9 days	4 days	6 days	9 days
1	2 000	35 700 000	1 192 000 000	0	80	0
2	3 000	12 000 000	60 000 000	0	2 000	0
3	8 000	26 600 000	391 000 000	0	0	0
4	1 000 000	63 280 000	45 360 000	0	900	0
5	240 000	7 048 000	43 000 000	0	0	0
6	0	24 000	0	0	0	0
7	0	0	12 000 000	0	0	0
8	0	0	800	0	0	0
9	1 000	21 560 000	ND	0	0	ND

**Pseudomonas infections:**

*Pseudomonas aeruginosa* appears to need excessively wet conditons for survival and the production of infections on normal skin. Survival of an inoculum of  $10^5$  cells under occlusion with polyethylene film was detected in only 5 of 12 sites. We recently discovered that applying a water soaked patch of non-woven cotton (Webril) would regularly induce within 5 to 8 days a vivid dermatitis from which large numbers of *Pseudomonas* could be recovered. We seem to have hit on a useful model in humans.

**Methods:** In 9 subjects one forearm was wrapped with polyethylene film held in place with adhesive tape while the other forearm received a strip of wet felted cotton as well as the polyethylene occlusive dressing. The dressings were changed daily.

**Results:** All 9 subjects acquired spontaneous *Pseudomonas* colonization of the arm treated with wetted cotton felt under occlusion while only 3 showed any *Pseudomonas* on the arm sample occluded. A rash developed within 9 days in 6 of the 9 subjects. This model will be further investigated.

## DOCUMENT CONTROL DATA - R &amp; D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Department of Dermatology Hospital of the University of Pennsylvania 3600 Spruce Street, Philadelphia, Penna. 19104		2a. REPORT SECURITY CLASSIFICATION	
		2b. GROUP	
3. REPORT TITLE SUSTAINED PROTECTION AGAINST SUPERFICIAL BACTERIAL AND FUNGAL INFECTIONS BY TOPICAL TREATMENT (U)			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) ANNUAL PROGRESS REPORT 1 <sup>Sept</sup> 1971 - 31 May 1972			
5. AUTHOR(S) (First name, middle initial, last name) Albert M. Kligman, M. D., Ph. D. Richard R. Marples, B. M., M. Sc., M. R. C. Path.			
6. REPORT DATE August 1972	7a. TOTAL NO. OF PAGES 18	7b. NO. OF REFS	
8a. CONTRACT OR GRANT NO. DADA-17-71-C-1009	8b. ORIGINATOR'S REPORT NUMBER(S)		
8c. PROJECT NO.			
9.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)		
4.			
10. DISTRIBUTION STATEMENT Distribution limited to U. S. Government agencies only, proprietary information; June 1972. Other requests for this document must be referred to Commanding General, U. S. Army Medical Research and Development Command.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY	
13. ABSTRACT  The feasibility of providing sustained <u>protection of the skin against infection</u> by common micro-organisms has been shown in previous reports. Some <u>anti-microbial</u> agents remain active and persistent on human skin. <u>Neomycin</u> reduces growth of the flora under occlusion at a dose of 0.4 micrograms per sq. cm. <u>Neomycin</u> , <u>chloramphenicol</u> and some other drugs will reduce the high numbers of organisms created by preocclusion.  <u>Experimental infections with Staphylococcus aureus</u> on mildly traumatized skin have been extensively investigated. By delaying inoculation of the pathogen until 24 hours after injury a safe yet realistic infection can be produced. The requirements for a valid test of antibacterial action are still in doubt but the ability of the test to detect effective antibacterial action is not doubted. Similarly basic studies of the <u>Candida albicans</u> infection model have indicated the safety repeatability and practical value of this test. Infections with <u>Pseudomonas</u> can now be created but repeatability remains a problem.			

DD FORM 1473 1-73



DEPARTMENT OF THE ARMY  
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND  
504 SCOTT STREET  
FORT DETRICK, MARYLAND 21702-5012

REPLY TO  
ATTENTION OF:

MCMR-RMI-S (70-1y)

6 Jun 00

MEMORANDUM FOR Administrator, Defense Technical Information  
Center, ATTN: DTIC-OCA, 8725 John J. Kingman  
Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statements

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Contract Numbers DA-49-193-MD-2137 and DADA17-71-C-1099. Request the limited distribution statements for Accession Document Numbers **AD807333L, AD839773L, AD858422L, AD874484L, AD888196L, AD903316L, and ADB002516**, be changed to "Approved for public release; distribution unlimited." This report should be released to the National Technical Information Service. }

2. Point of contact for this request is Ms. Virginia Miller at DSN 343-7327 or by email at Virginia.Miller@det.amedd.army.mil.

FOR THE COMMANDER:

*Phyllis Rinehart*  
PHYLIS M. RINEHART  
Deputy Chief of Staff for  
Information Management

- Verify the document  
- Ask more than one  
unique identifier  
JRM